Claims

1. Process for the production of a eukaryotic alkaline phosphatase in yeast cells comprising the steps:
   a) cloning a gene sequence into different vectors
   b) transformation of the yeast, c) expression and
   d) purification of the alkaline phosphatase, wherein
   - a first vector has a resistance gene for a first selection marker
   - transformants which have integrated the resistance gene and the gene sequence into the genome are selected by growth on nutrient medium containing a low concentration of a first selection marker,
   - the gene copy number is increased by multiple transformation in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure,
   - a second vector is added which has a resistance gene for a second selection marker,
   - the gene copy number is increased by multiple transformation with the second vector in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure and
   - those clones are selected which have integrated several copies of the gene sequence and the selection marker resistance genes in a stable manner.

2. Process according to the invention, wherein the gene sequence corresponds to SEQ ID NO:1.

3. Process as claimed in one of the claims 1 or 2, wherein the gene sequence corresponds to SEQ ID NO:5.

4. Process as claimed in one of the claims 1 to 3, wherein methylotrophic yeast cells are used.

5. Process as claimed in one of the claims 1 to 4, wherein Pichia pastoris or Hansenula polymorpha is used as the yeast strain.

6. DNA according to SEQ ID NO:5.

7. Vector containing SEQ ID NO:5.

8. Vector as claimed in claim 7, which essentially corresponds to pHAP10-3.

9. Vector containing the entire expression cassette from pHAP10-3.

10. Vector as claimed in claim 9, which essentially corresponds to pHAP10-3/9K.

11. Host strain transformed with a vector as claimed in claim 9 or 10.

12. Host strain transformed with the vector pHAP10-3/9K and/or a vector as claimed in claims 7 or 8.

13. Host strain as claimed in claim 12, wherein Pichia pastoris or Hansenula polymorpha is used as the host strain.

14. Pichia pastori X-33 strain transformed with a vector as claimed in claims 8 to 10.

15. Process for producing a eukaryotic highly active alkaline phosphatase, wherein the enzyme is expressed in a host strain as claimed in one of the claims 11 to 14.

Cl' cancel

add Cl)